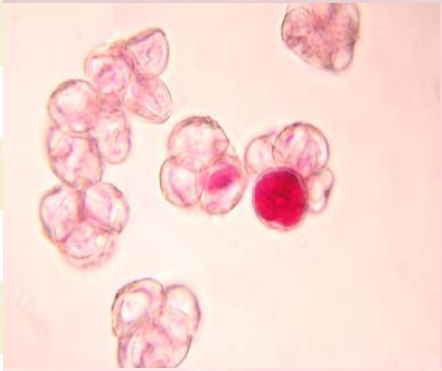
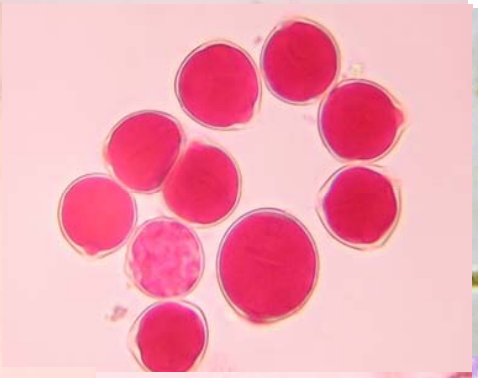
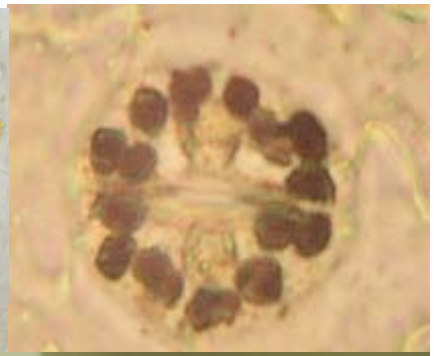
MANUAL TÉCNICO

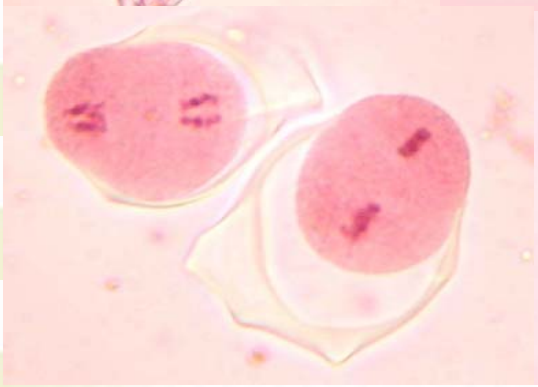
Biología reproductiva y Citogenética de la papa

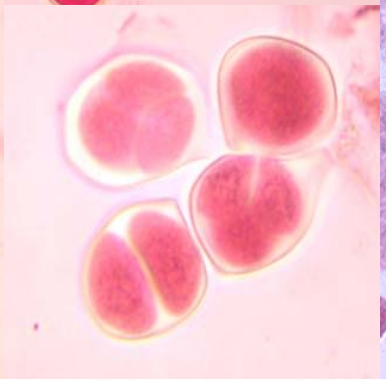
M. Orrillo * M. Bonierbale

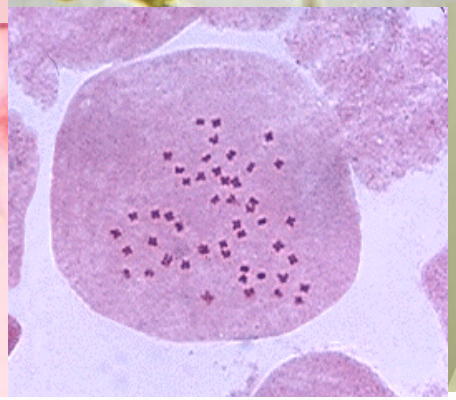












Evaluación de la Ploidía en *Solanum spp.*

CIP- Laboratorio de Citogenética

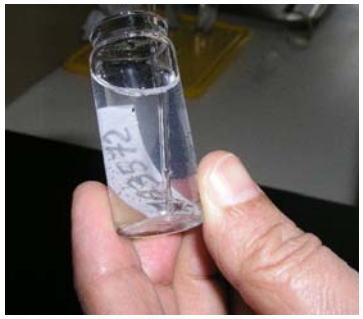
CONTEO DE CROMOSOMAS

PROCEDIMIENTO

Colección de raíces en:
(H_2O destilada + 15*u*L Permetrina, pH 5-5.8)

24 horas

Eliminar la solución de pre-fijación



Hidrólisis con HCl 1 N previamente precalentado

8-10 min a 60^0C

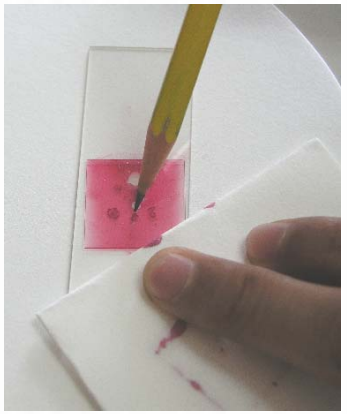
Eliminar la solución & Adicionar H_2O destilada

Colocar las raíces en Placa Petri

Tinción con Lacto-Propiónico Orceína (15min aprox.)

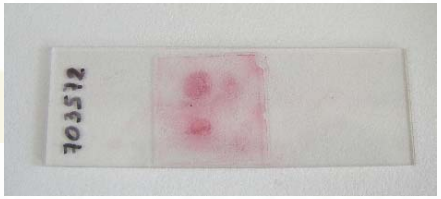
Cortar 1-2mm de la punta de las raíces

Presionar para extender el tejido

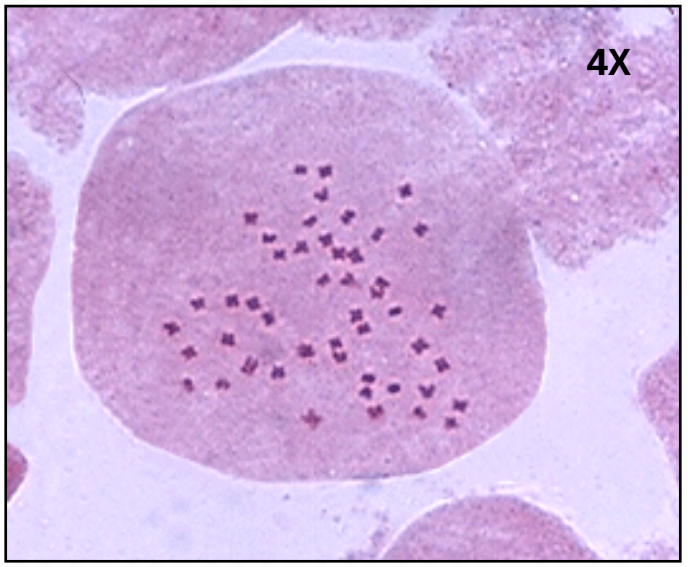


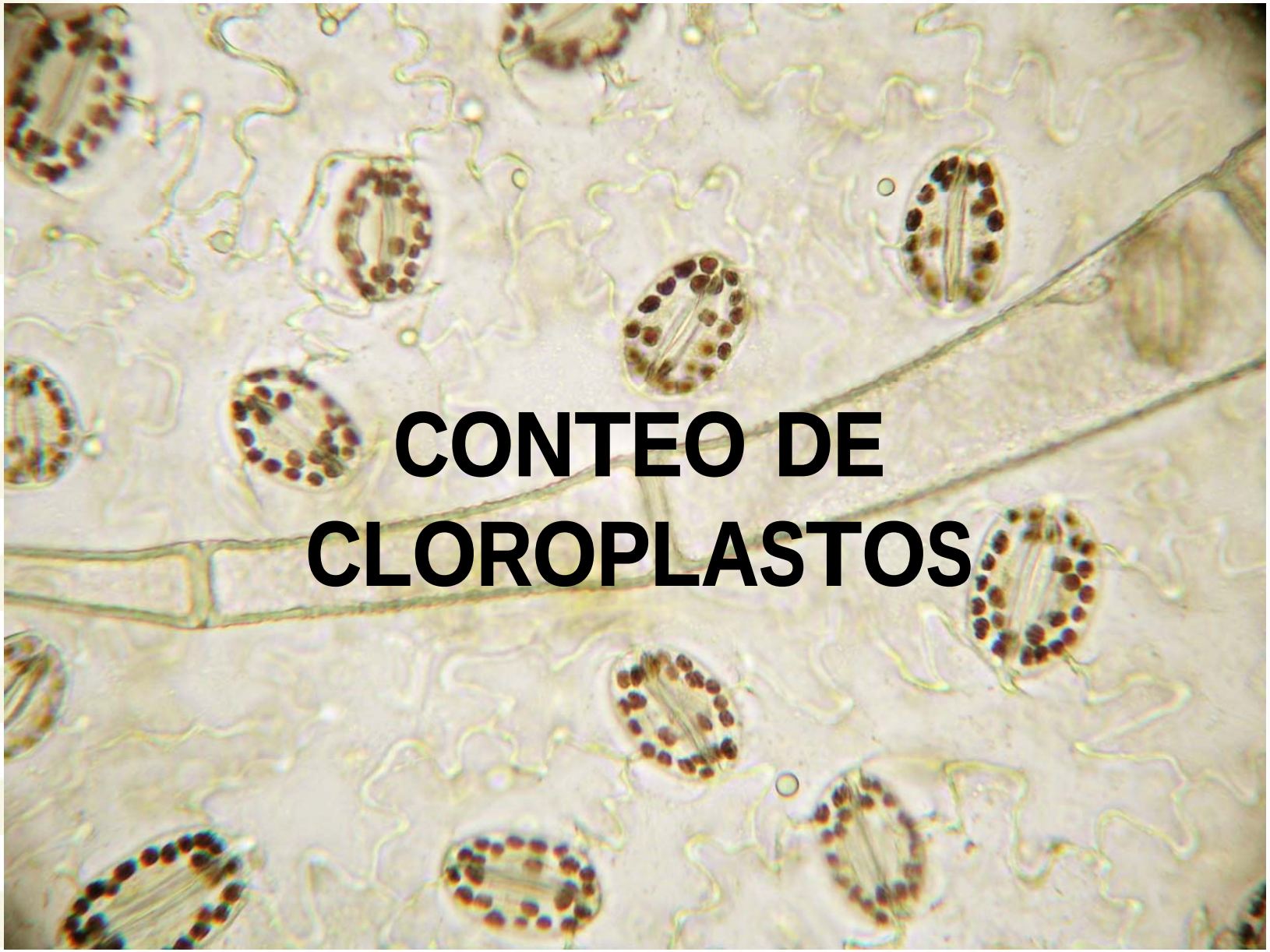
Aplastado
(Squash)

Lámina lista



Observar al microscopio

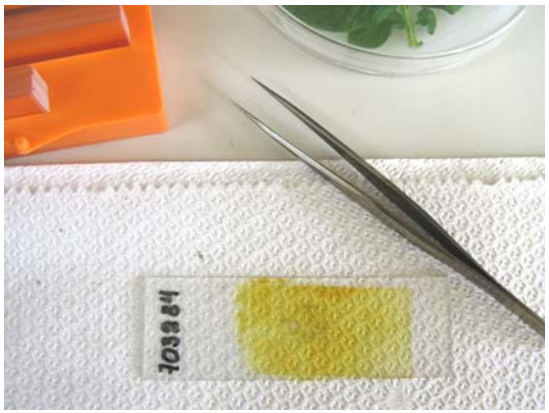



CONTEO DE
CLOROPLASTOS

PROCEDIMIENTO

Colecta de hojas, foliolos

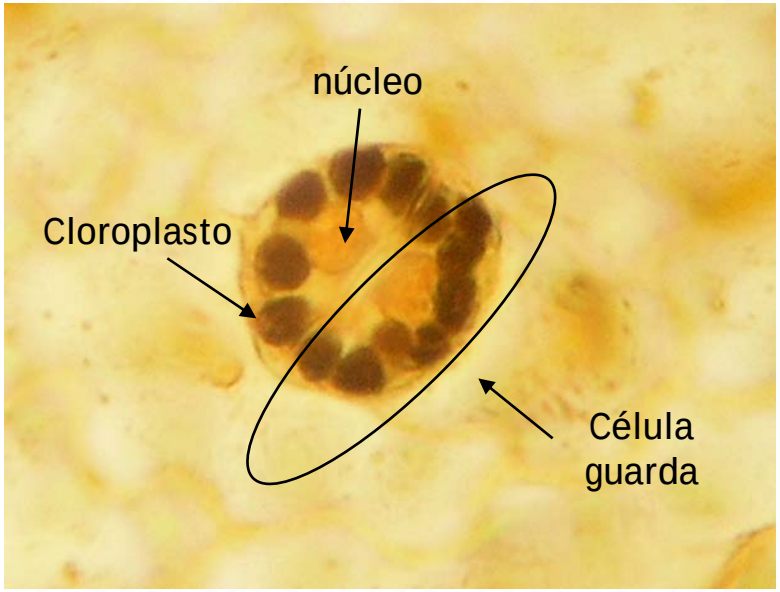
Obtención del tejido epidérmico del envés usando una pinza fina



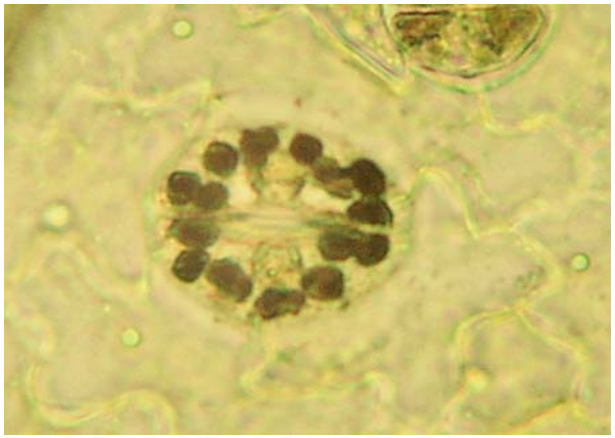
Muestra lista para su observación

Promedio del N ° de cloroplastos por célula guarda	Posible ploidía
7 - 8	2X
9 - 11	3X
12 -14	4X
15 -16	5X

Huamán, 1995



Contar los cloroplastos en 10 células guarda, de tamaño similar y solo una célula por estoma



Genotipo Diploide

Posiblemente Genotipo Tetraploide

ANÁLISIS DE PLOIDÍA EN EL CITOMETRO DE FLUJO

Citometría de Flujo

Ploidy Analyzer (PA)
Partec I

MATERIALES

partec
CyStain UV Ploidy
DAPI Staining Solution
Store at 4° C in the dark
Order No.: 05-5001
LOT No.: w0050426
expiry 06/06
500 ml

PROCEDIMIENTO

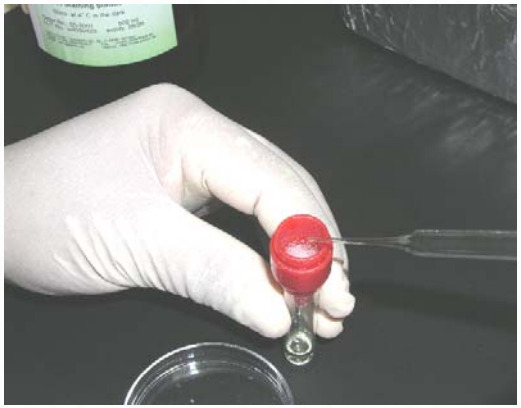
Colectar aprox. 50mg hojas jóvenes apicales

Cortar solo los foliolos

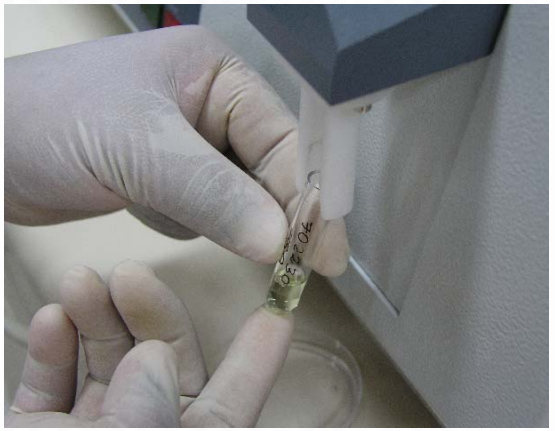
Colocarlos en 0.5 ml el buffer de extracción (Cystain UV ploidy)

Picar muy suavemente y de arriba hacia abajo

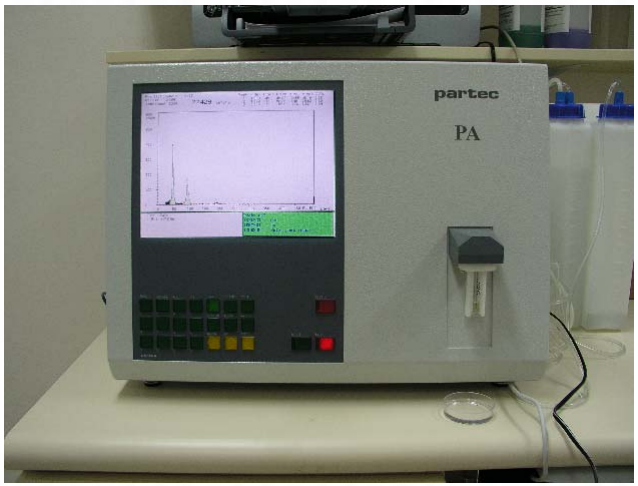
Adicionar 1.5ml de CyStain UV ploidy e incubar por 5 minutos



Filtrar la muestra en filtros de 30µm
(Partec Cell Trics 30)



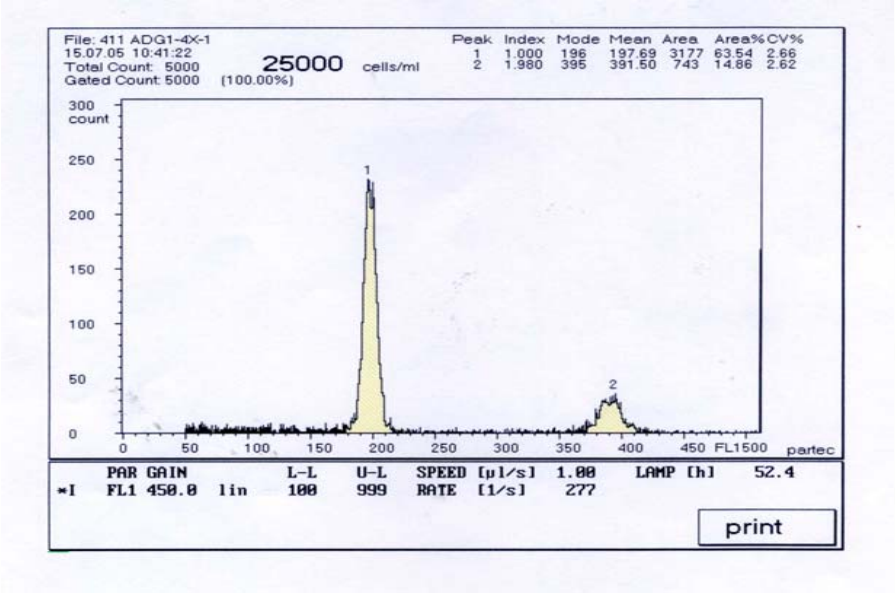
Colocar la muestra en
el Clitómetro de Flujo



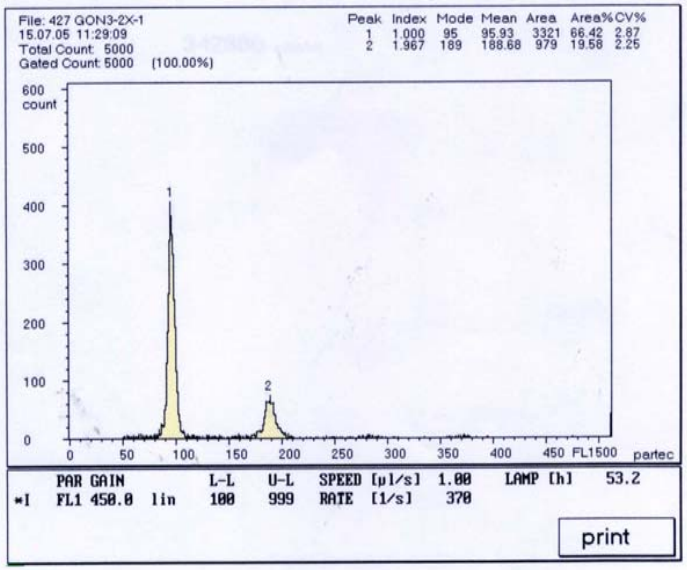
Citómetro de Flujo
Ploidy Analyzer (PA) Partec I

Se establece los parámetros para la lectura de la muestra

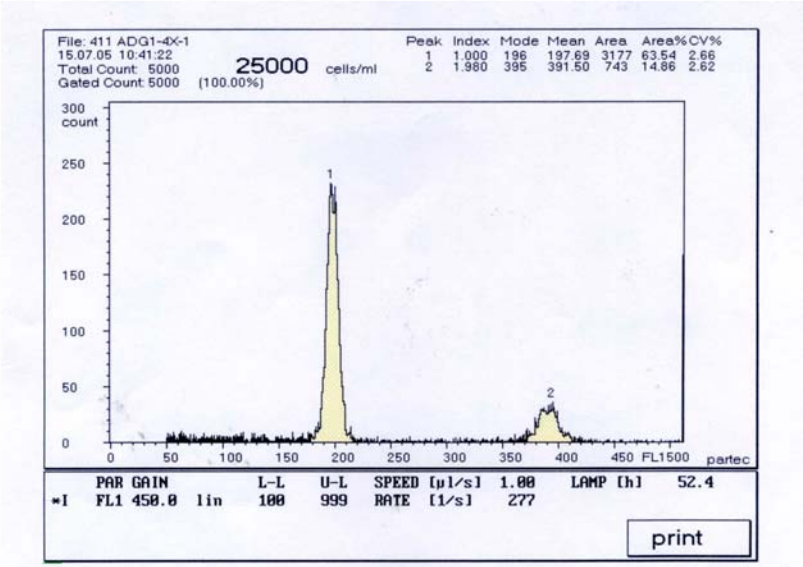
Histograma de la muestra



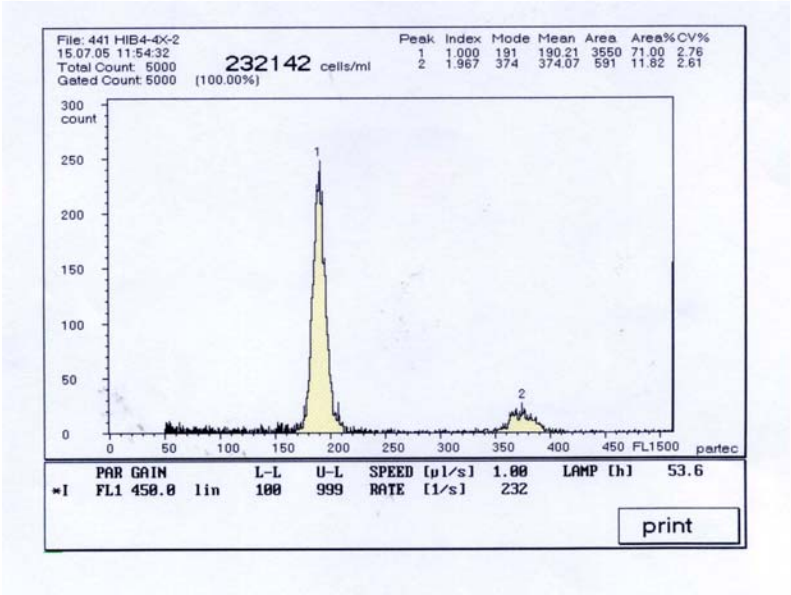
2x


File: 427 GON3-2X-1
15.07.05 11:29:09
Total Count: 5000
Gated Count: 5000 (100.00%)
Peak Index Mode Mean Area Area% CV%
1 1.000 95 95.93 3321 66.42 2.87
2 1.967 189 188.68 979 19.58 2.25
600 count
500
400
300
200
100
0
0 50 100 150 200 250 300 350 400 450 FL1500
partec
PAR GAIN L-L U-L SPEED [µl/s] 1.00 LAMP [h] 53.2
*I FL1 450.0 lin 100 999 RATE [1/s] 370
print

4x


File: 411 ADG1-4X-1
15.07.05 10:41:22
Total Count: 5000
Gated Count: 5000 (100.00%)
25000 cells/ml
Peak Index Mode Mean Area Area% CV%
1 1.000 196 197.69 3177 63.54 2.66
2 1.980 395 391.50 743 14.86 2.62
300 count
250
200
150
100
50
0
0 50 100 150 200 250 300 350 400 450 FL1500
partec
PAR GAIN L-L U-L SPEED [µl/s] 1.00 LAMP [h] 52.4
*I FL1 450.0 lin 100 999 RATE [1/s] 277
print

< 4X


File: 441 HIB4-4X-2
15.07.05 11:54:32
Total Count: 5000
Gated Count: 5000 (100.00%)
232142 cells/ml
Peak Index Mode Mean Area Area% CV%
1 1.000 191 190.21 3550 71.00 2.76
2 1.967 374 374.07 591 11.82 2.61
300 count
250
200
150
100
50
0
0 50 100 150 200 250 300 350 400 450 FL1500
partec
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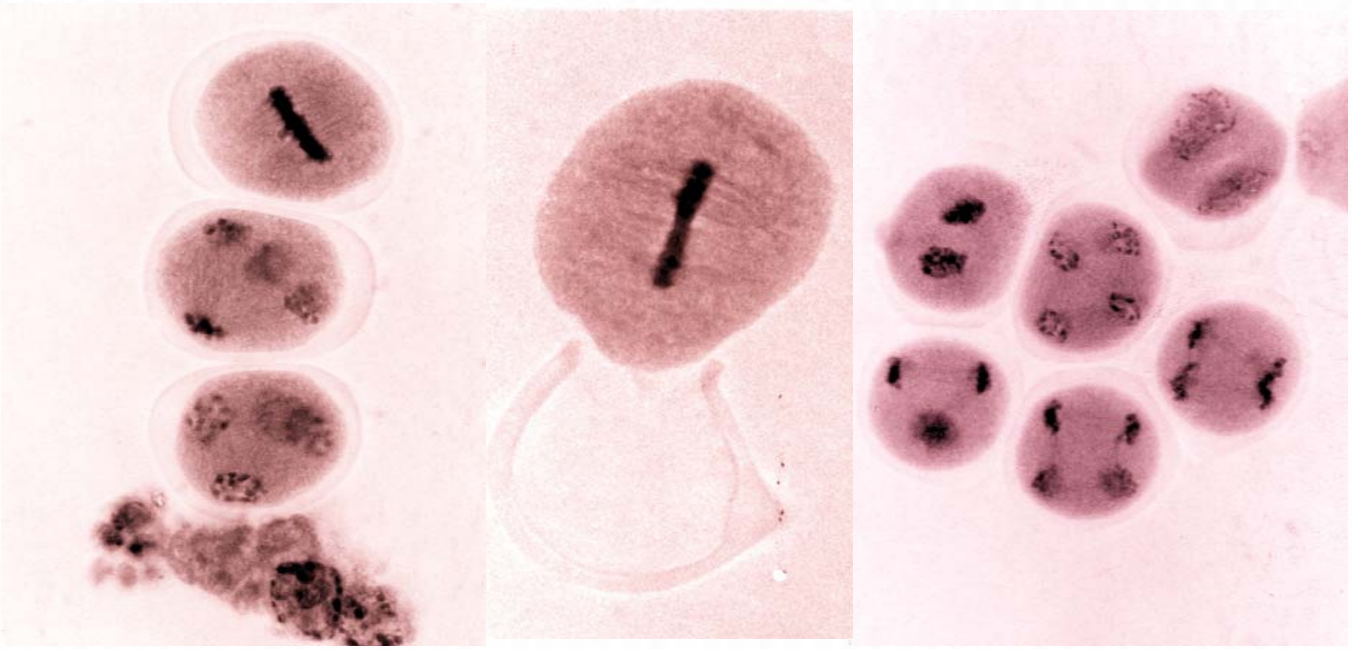
*I FL1 450.0 lin 100 999 RATE [1/s] 232
print

MEIOSIS



PROCEDIMIENTO

Botones florales recolectados en Fijador Carnoy Modificado

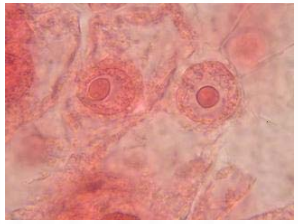
Anteras en alcohol de 70% colocadas en placa Petri listas para su disección

Lámina lista para su observación

Antera diseccionada

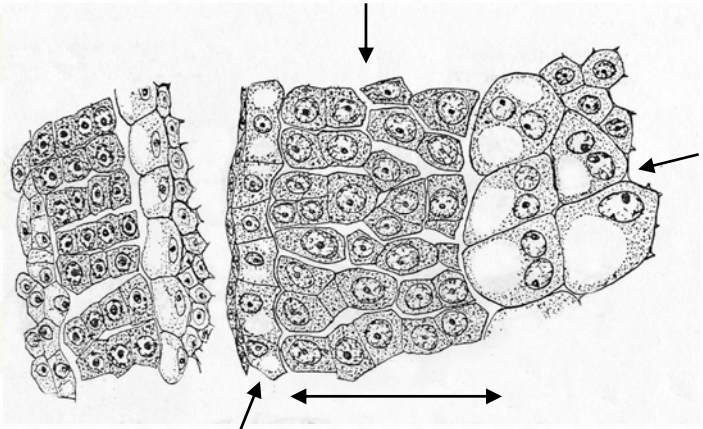
Microsporogénesis

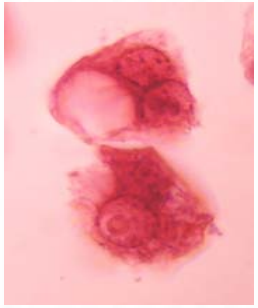
Células madre del polen

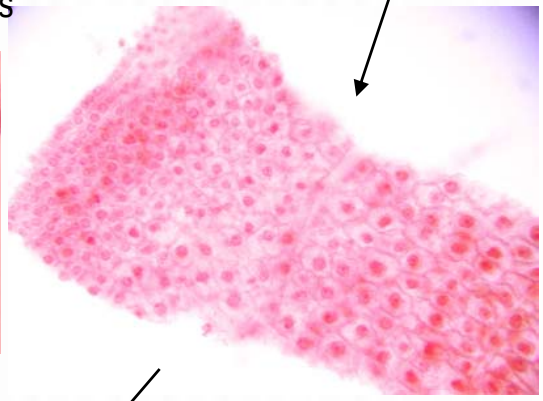


Células madre del polen

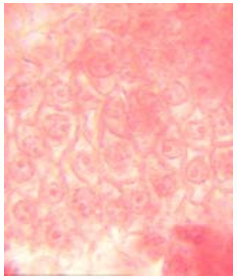
Células nutritivas



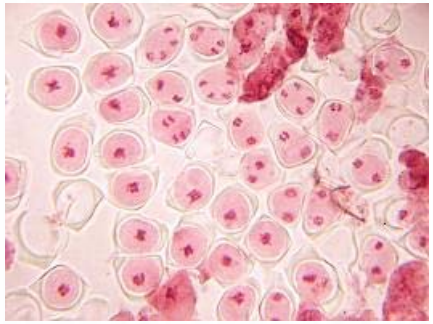




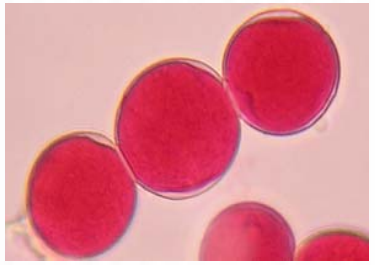
Arquesporio



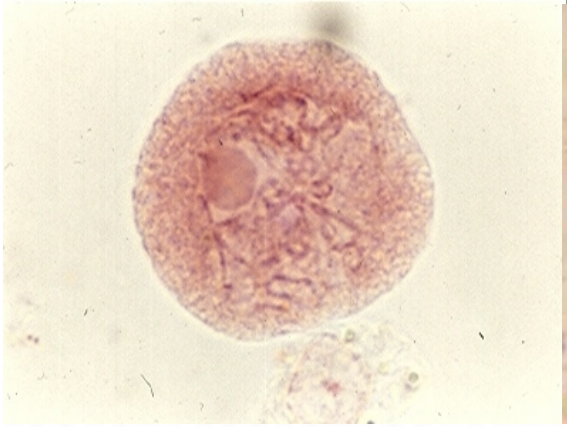
Células del Tapetum



Meiosis



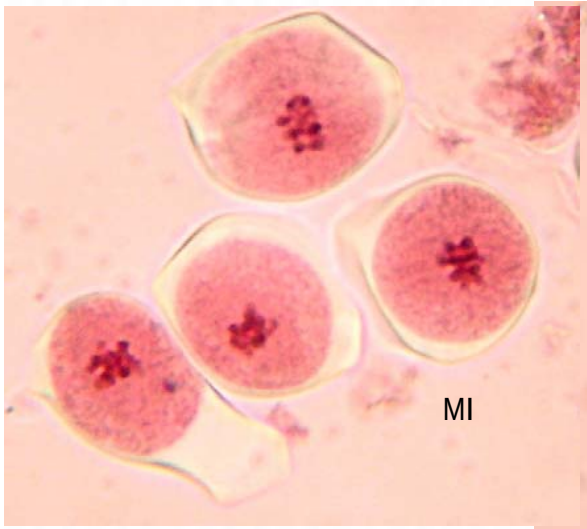
Polen



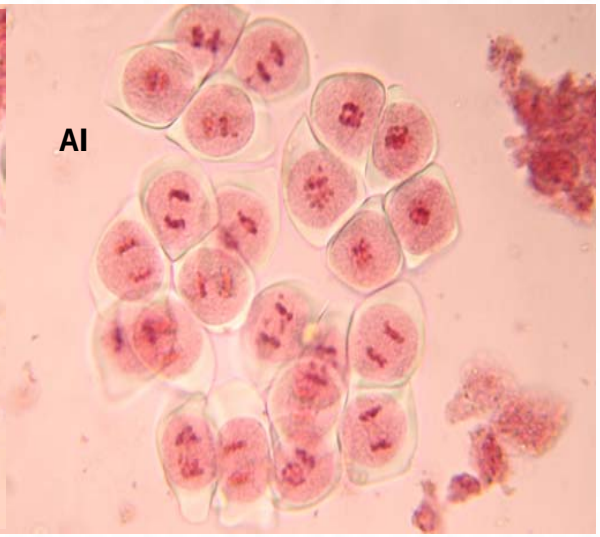
Paquiteno

Diacinesis

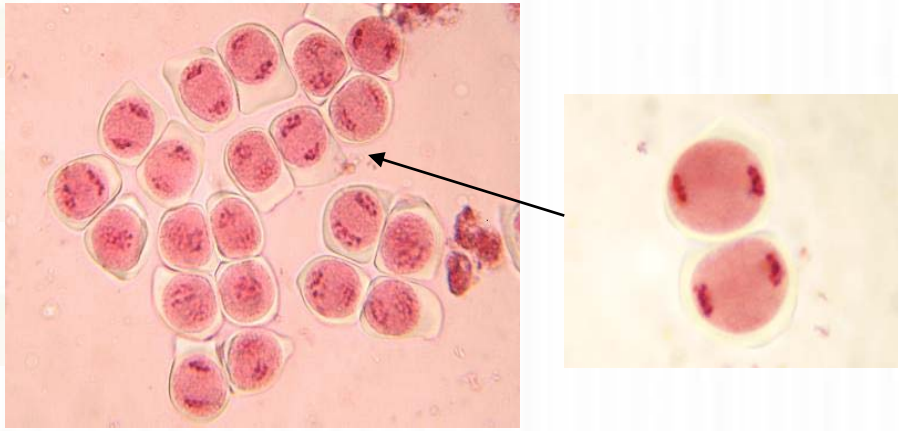
Profase I



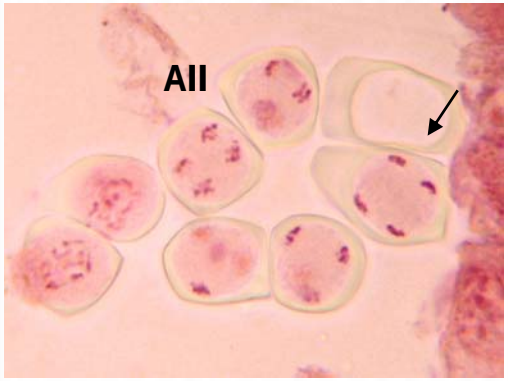
Metafase I



Anafase I

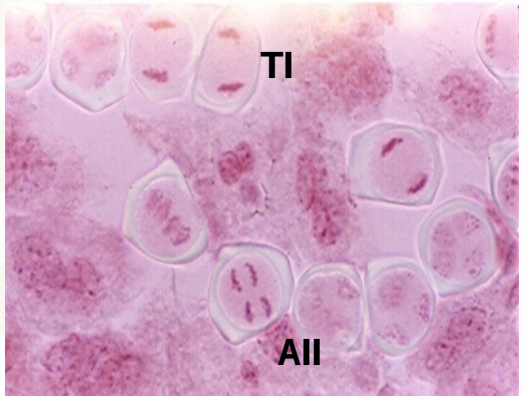


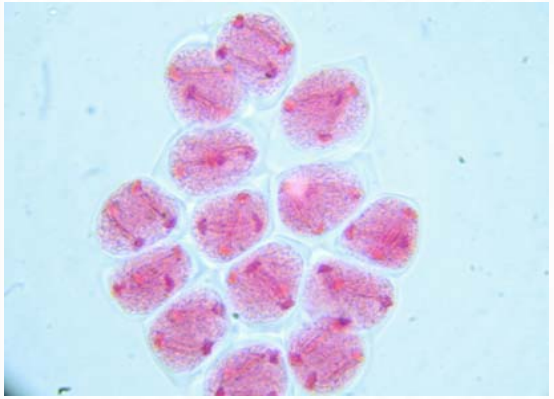
Telofase I



Metafase II, ejes de los spindles (flecha) son paralelos

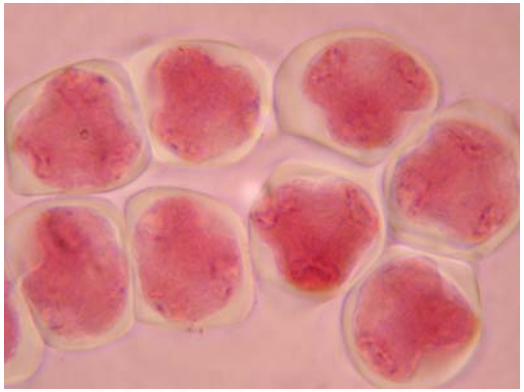
Anafase II, ps y telofase temprana II (flecha)



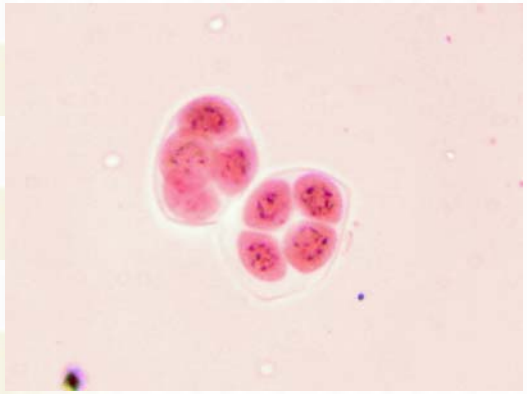


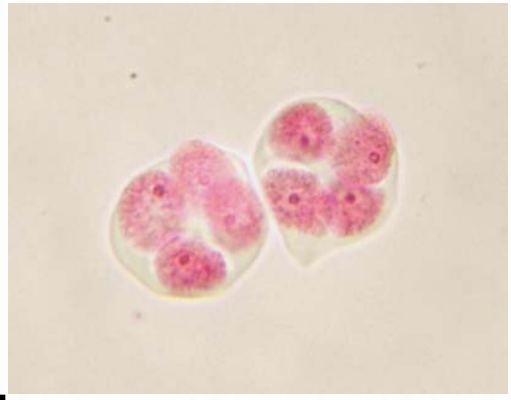
Telofase I, Anafase II

Anafase II, husos normales y paralelos

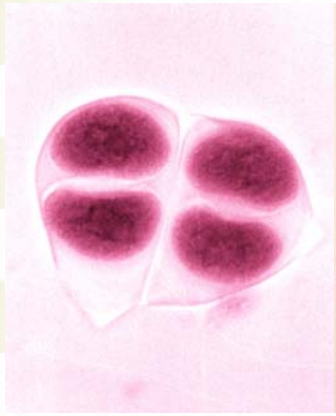


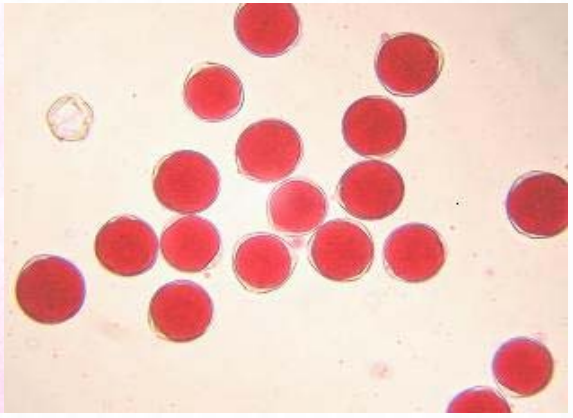
Formación de las constricciones que separan las microsporas





Producto final de las dos divisiones meióticas: Cuatro microsporas y sus núcleos, pero aún encerradas en la pared original

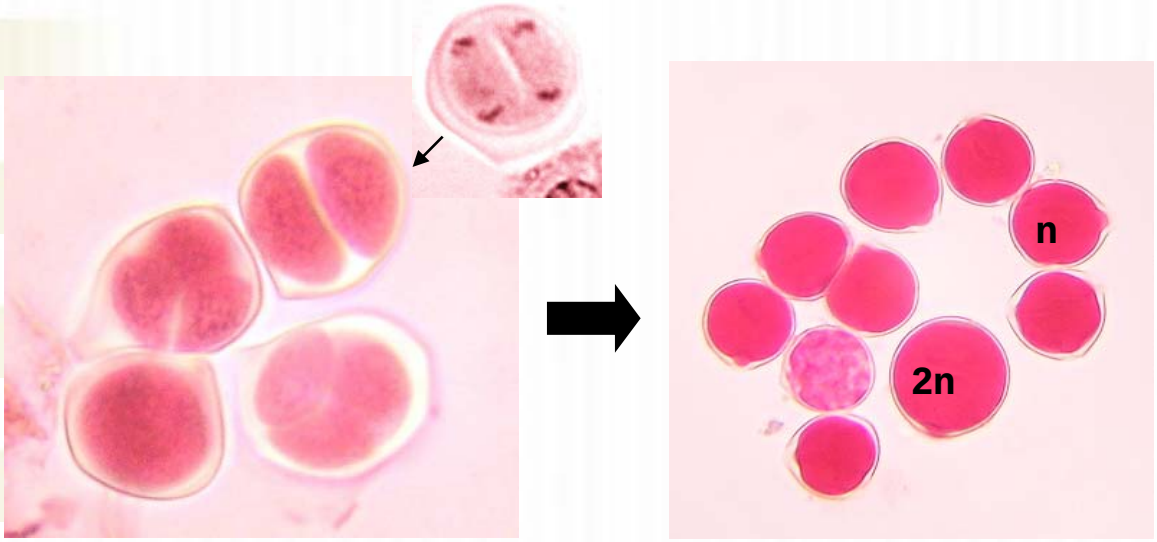




Tétradas

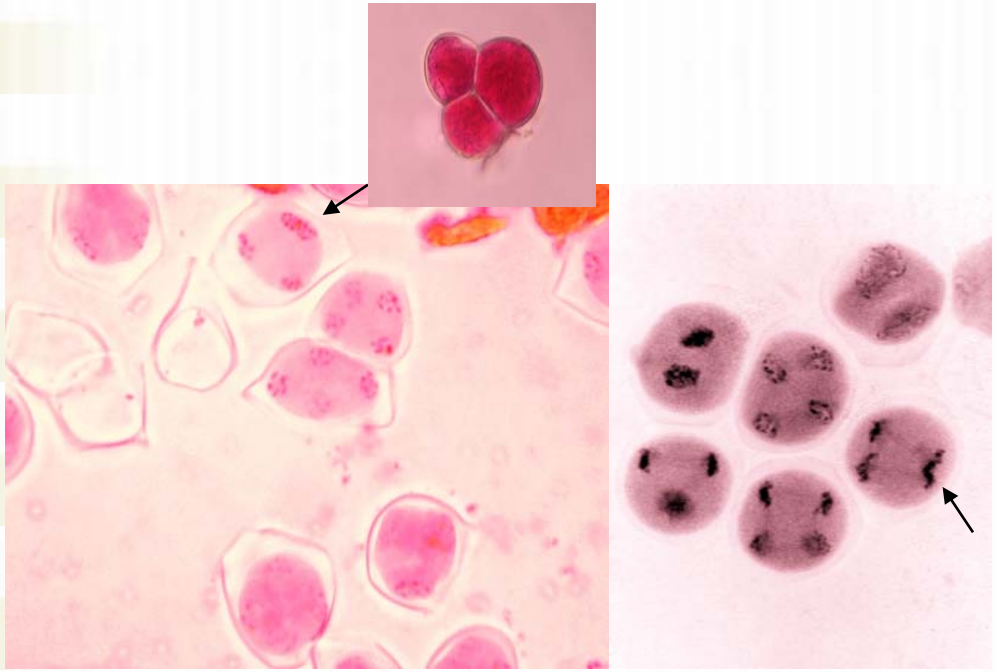
Y

Granos de polen ya liberados



Formación de tétradas y diadas

Formación de polen normal y polen 2n



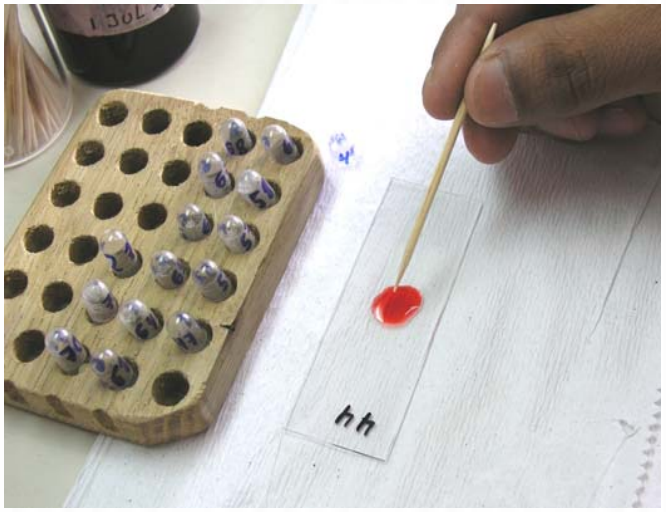
Formación de triadas (orientación tripolar) y tétradas

EVALUACIÓN DE LA VIABILIDAD DEL POLEN

PROCEDIMIENTO

Colección de polen

Polen colectado en cápsula de gelatina



La muestra es colocada en 2 - 3 gotas de colorante

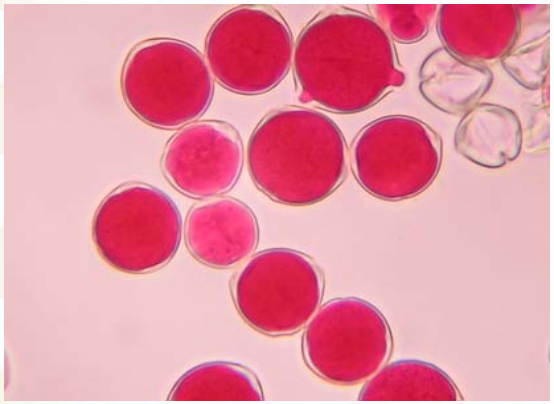
Materiales

La muestra es cubierta con un cubre-objeto

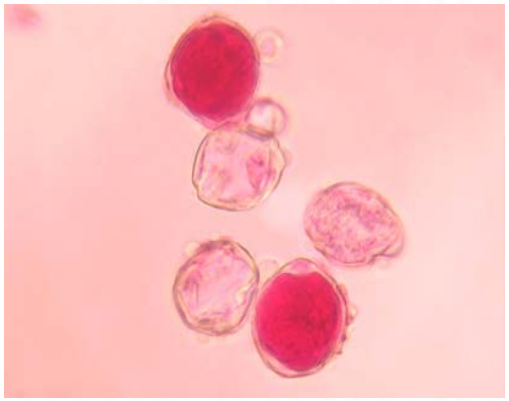
Las muestras son colocadas en posición horizontal

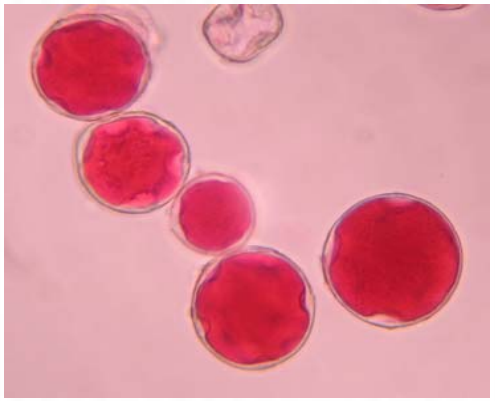
COLORANTE: Gelatina Aceto-carmín Glicerol

COLORACIÓN + polen bien formado= VIABILIDAD

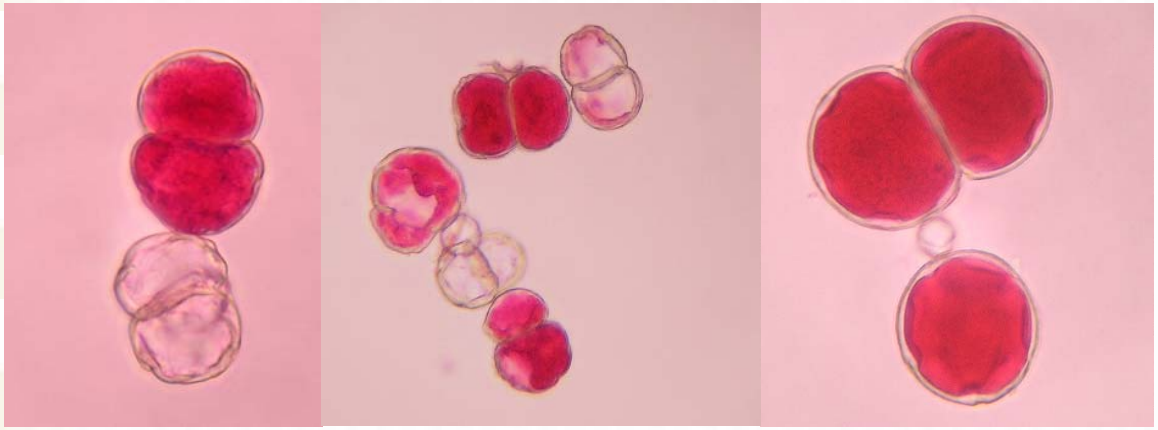


Polen normal (n) + polen estéril no coloreado

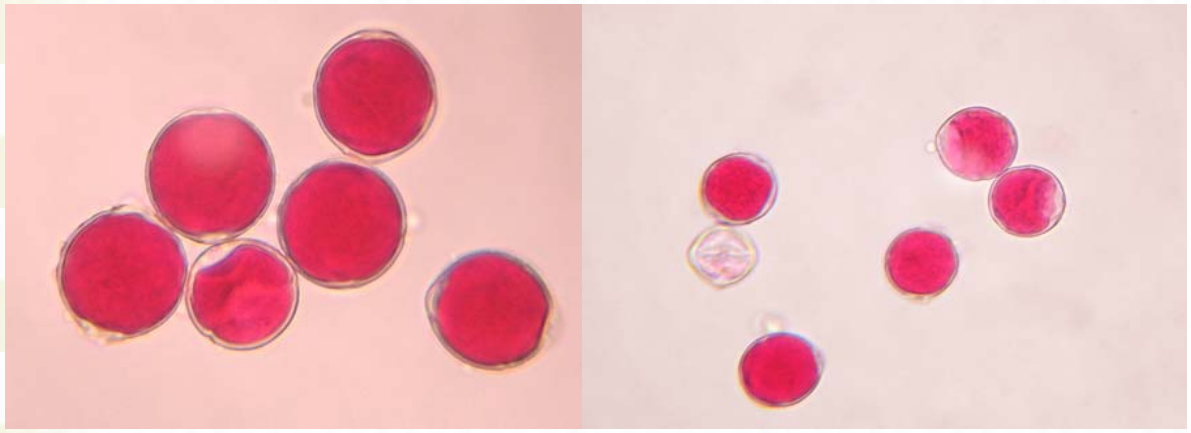




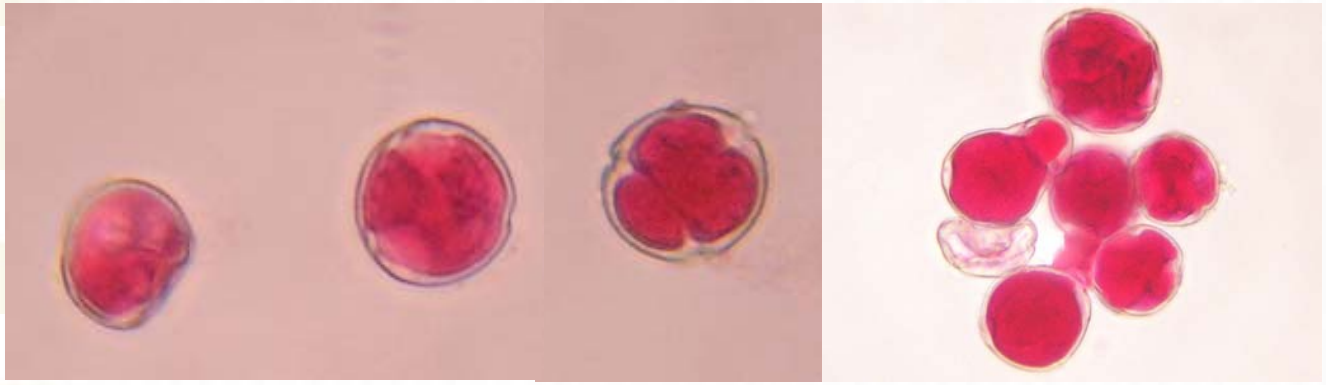
Polen estéril (n) coloreado y no coloreado



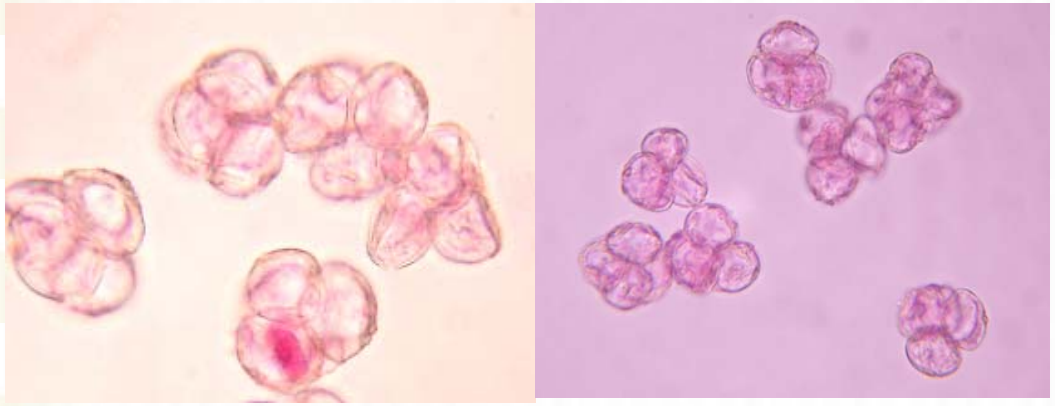
Polen doble, coloreado y no coloreado



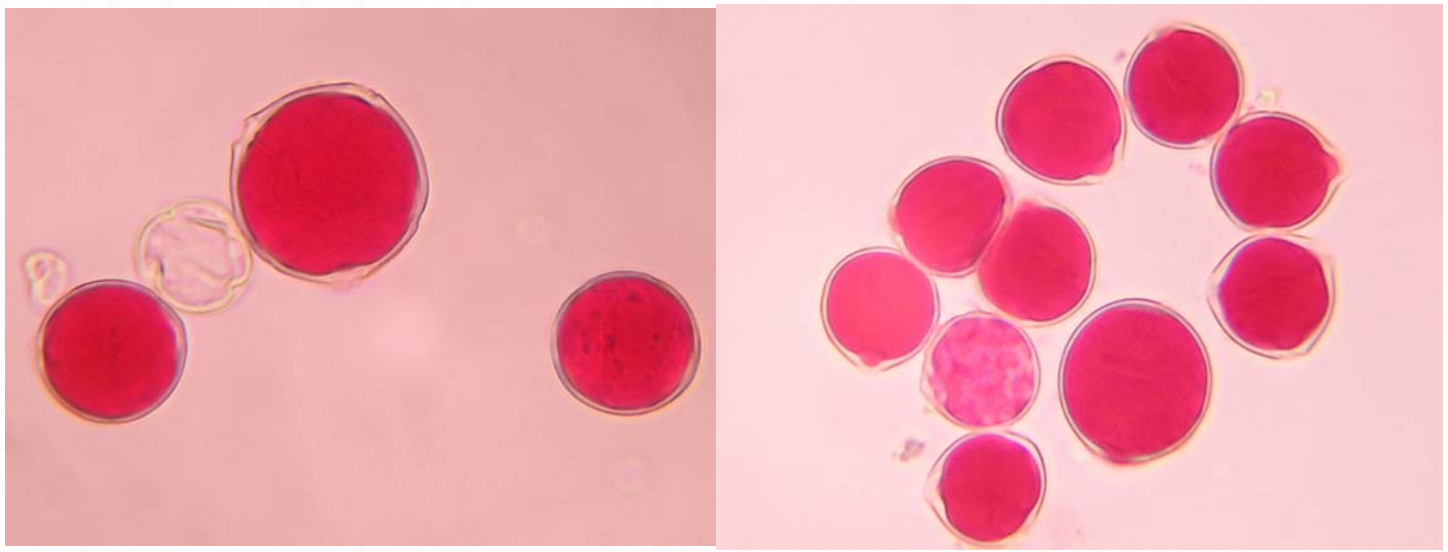
Eclipse de esterilidad



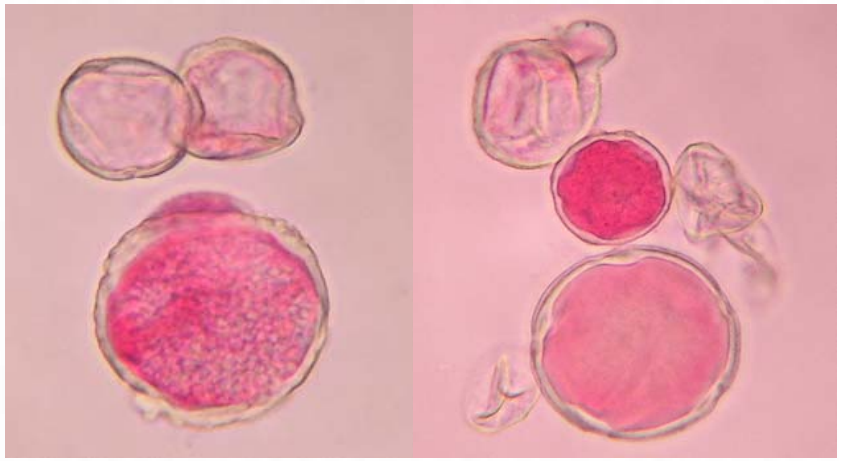
Polen anómalo



Tétradas estériles

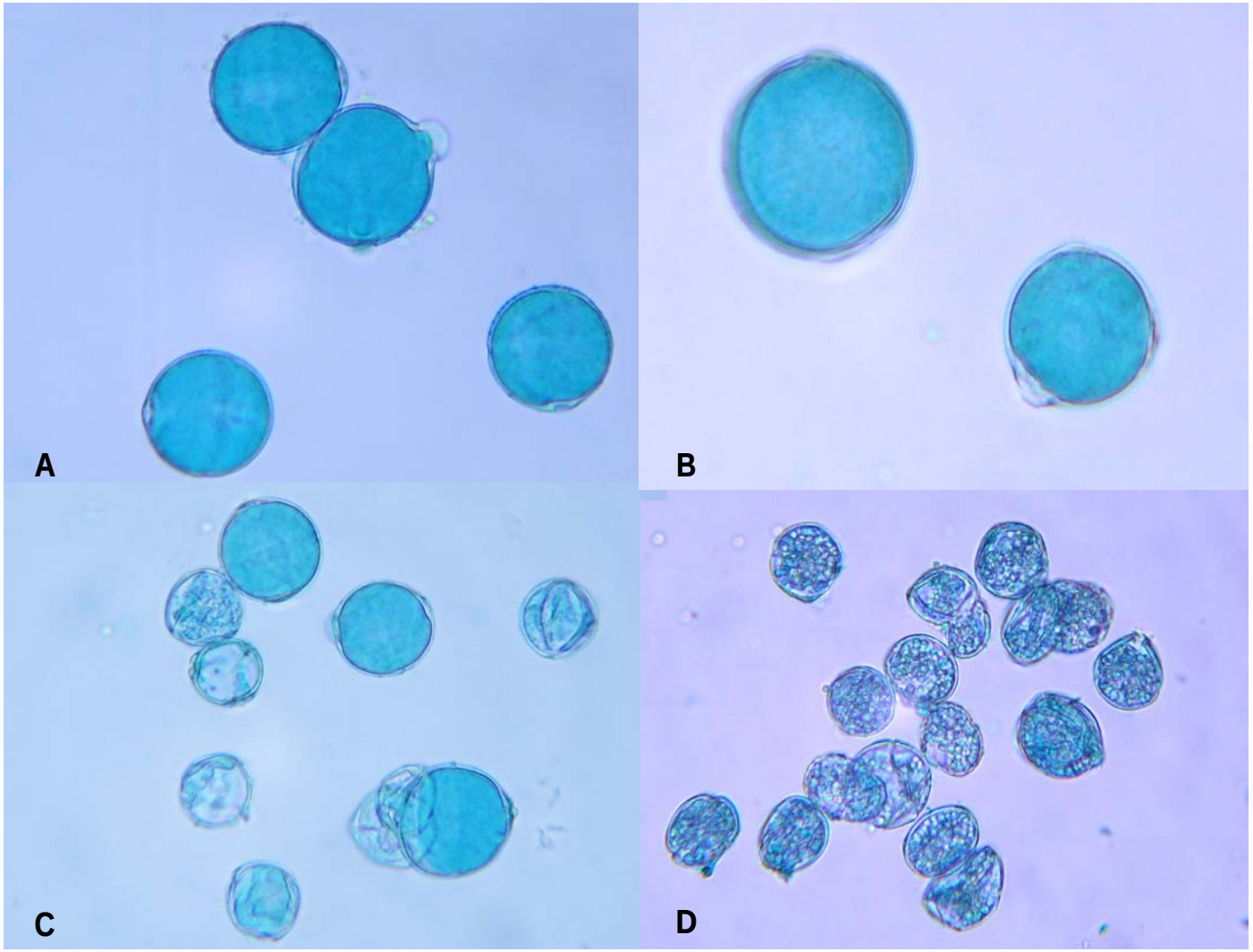


Polen n y 2n coloreados

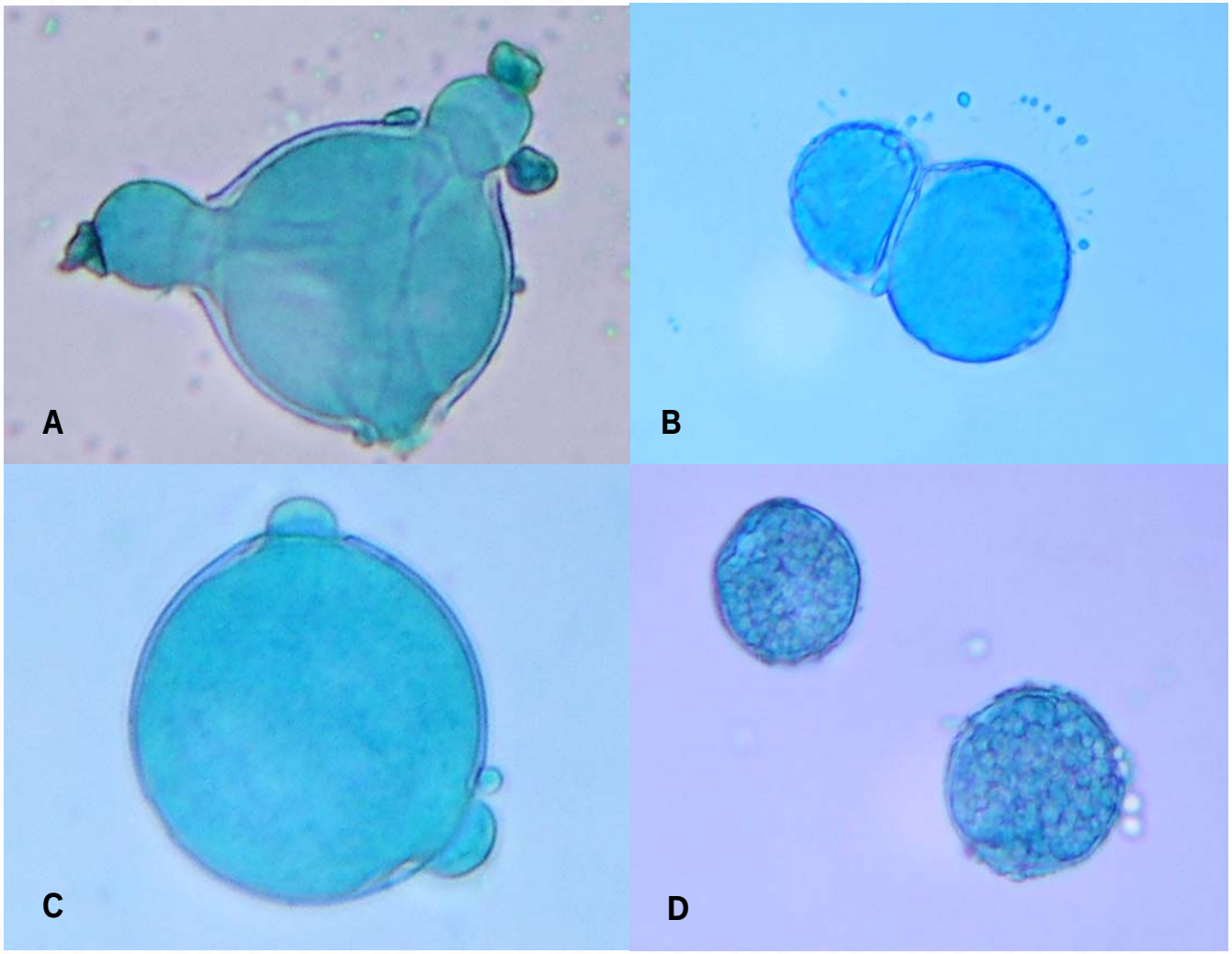


Polen n y 2n estériles

Polen: Coloración X-Gal



A. Polen normal (n), **B.** Polen n y 2n, **C.** Polen normal y estéril, **D.** Polen estéril



A. Polen con 3 poros germinativos, **B.** Polen doble, **C.** Polen con 2 poros germinativos **D.** Polen estéril

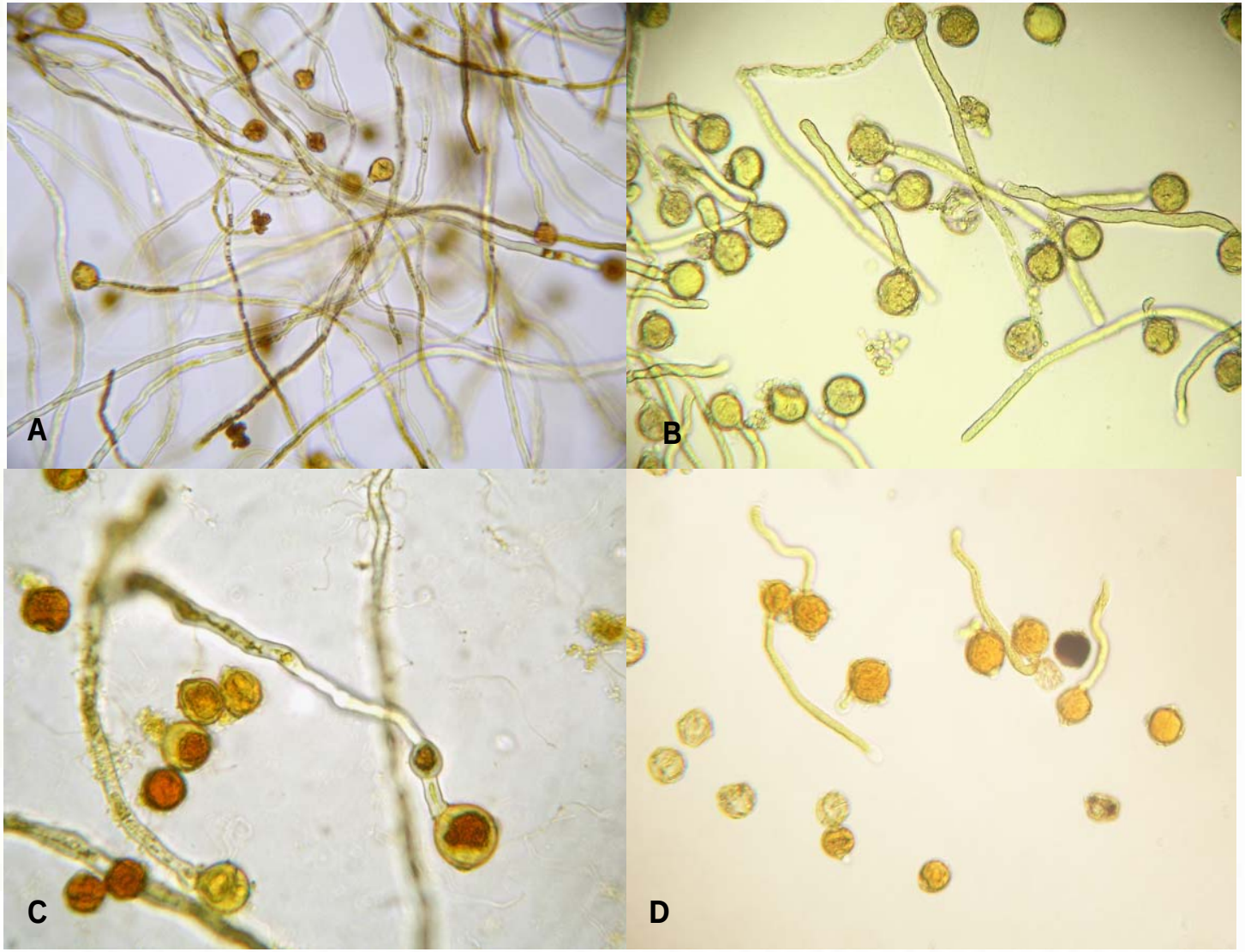
Cultivo de polen *in vitro*

PROCEDIMIENTO

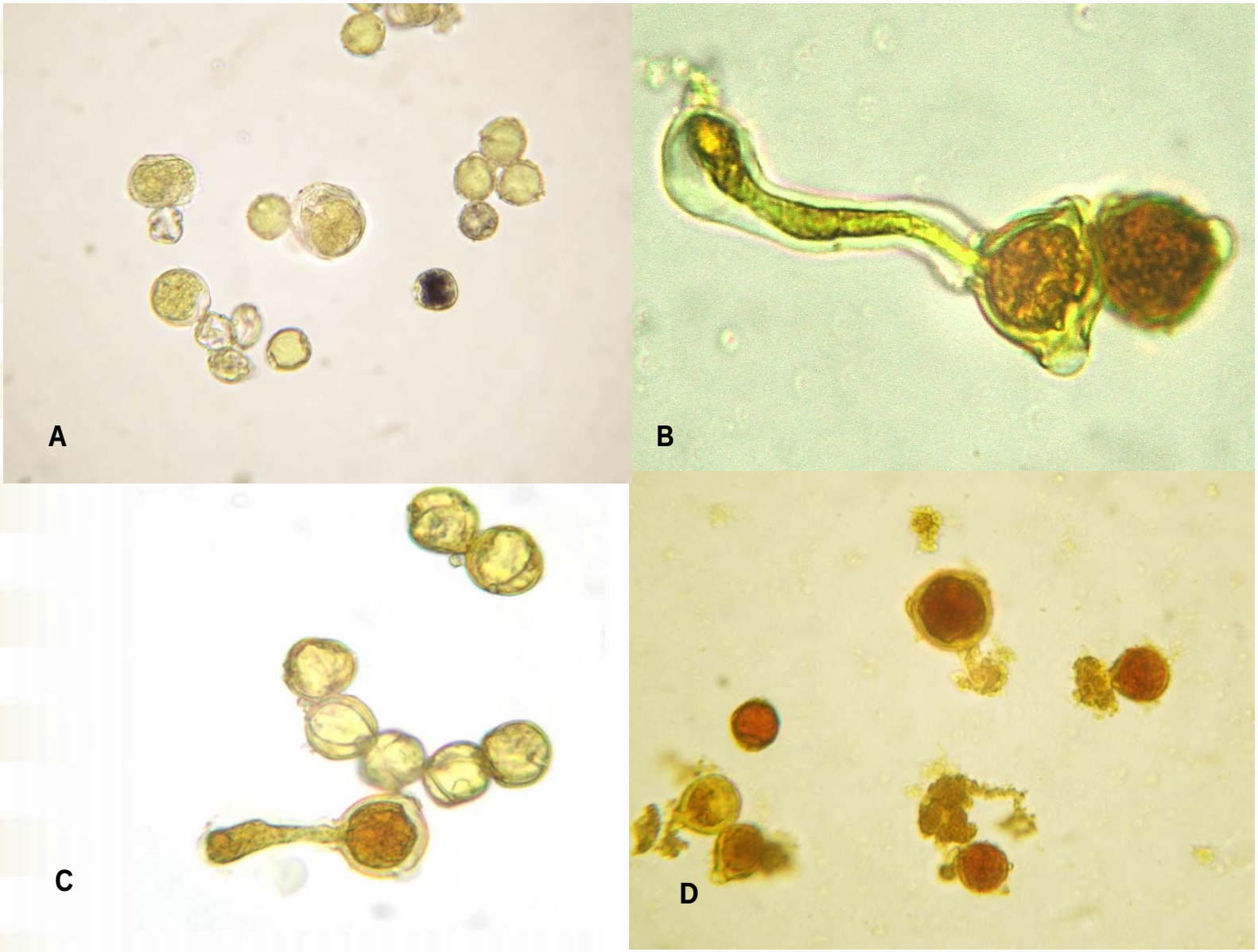
Materiales y medio de sucrosa al 20% + ácido bórico 200 ppm + Tween 20 (0.2%)

Siembra del polen en el medio de cultivo

Polen ya sembrado bajo cámara húmeda



A, B . Polen germinado y con buen crecimiento en el medio de cultivo. **C**. Polen con mediana germinación **D.** Polen con escasa germinación



A. Polen no germinado B. Polen doble germinando solo uno de los granos C. Escaso polen germinado D. Polen soltando su contenido

POLINIZACION

Y

FERTILIZACION

PROCEDIMIENTO

Colecta de polen

Emasculación de las anteras

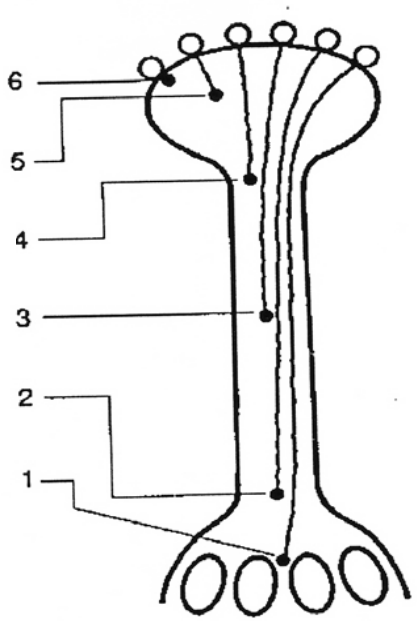
Guardar el polen en cápsulas de gelatina

Identificación del cruzamiento

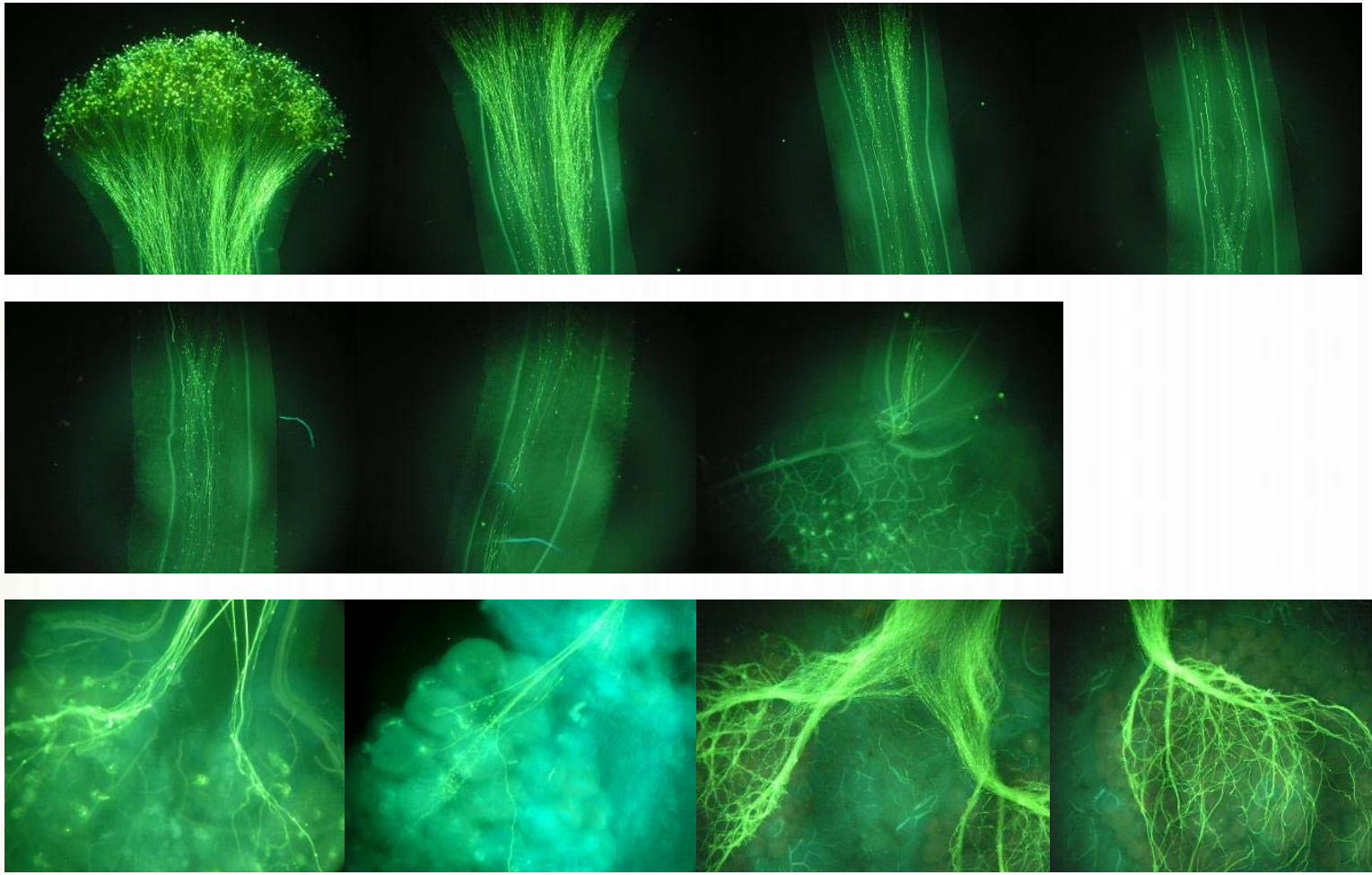
Polinización

Crecimiento del tubo polínico *in vivo* (en el pistilo)

Niveles*	Cantidad (número total de tubos polínicos en el estilo-ovario)		
	> 30	10-30	<10
	Valor combinado del nivel y cantidad de polen		
No germinado	16	17	18
Estigma	13	14	15
Estilo-estigma	10	11	12
Estilo	7	8	9
Estilo (final)	4	5	6
Placenta	1	2	3



Matriz de valores combinados para evaluación cualitativa (nivel de crecimiento al cual llegan los tubos polínicos en el pistilo) y cuantitativa (número de tubos polínicos llegando a los diferentes niveles a lo largo del pistilo) * Niveles de longitud del tubo del polen llegan: 6-Polen no germinado; 5 - estigma; 4 - estilo – estigma; 3-estilo; 2- final del estilo; 1 placenta. (Trognitz,1991).



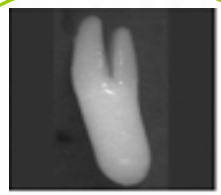
Diferentes vistas del crecimiento del tubo polínico en el pistilo después de ser coloreado con el colorante fluorescente azul de anilina el que reacciona con la callosa

RESCATE DE EMBRIONES

Polinización

Bayas listas para ser cosechadas (19-27 DDP)

Apertura de bayas *in vitro*



Torpedo

Globular **Corazón**

Rescate de pro embriones en diferentes estadíos

Descarte de embriones con marcador

Pro-embriones creciendo en medio de cultivo *in vitro*

Híbrido rescatado

Plantas de *in vitro* creciendo en invernadero

Propagación *in vitro*